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The Mechanism of Polymer Fractionation by Thin-Layer Chromatography

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The mechanism of thin-layer chromatographic (tlc) fractionation of polymeric substances by difference in molecular weight is examined. Two different mechanisms, one involves adsorption-desorption process and the other phase separation process, are considered. Some definitive evidences to affirm the mechanism due to the latter process are presented. However this does not rule out the possibility of the fractionation due to the former. Rather it is pointed out that fractionation in this mechanism ceases to be efficient decisively in the range of higher molecular weights (*e.g.* $>10^5$).

It is verified further that when a developer applied consists of polar and nonpolar solvents, phase separation of sample polymer occurs on chromatoplate for the following two reasons: The one is that a composition change along direction of development is produced spontaneously by selective adsorption of polar solvent components on adsorbent; the other is that the phase ratio, defined as the weight of developer retained per unit weight of adsorbent, decreases with increase of distance from dip level. In practical cases, the latter was found to be a more dominant cause for the phase separation. Thus one may attribute the main cause of fractionation by tlc to phase separation due to the decrease in the phase ratio. The phase separation *retards* migration of sample at a different rate which is larger for component with larger molecular weight, thus giving a smaller value of R_f (rate of flow) for a higher molecular-weight component. The shape of spots travelled on chromatogram and its variation with changing sample load are discussed also on the basis of phase separation mechanism. In addition some comments on the other factors affecting R_f -value are given.

I. INTRODUCTION

Separation of polymeric substances by molecular weight has been studied intensively because of its importance for practical as well as academic purposes in polymer chemistry. All of the fractionation methods with chromatographic techniques available to date belong, from the viewpoint of instrumental classification, to the category of column chromatography. While from that of separation mechanism, they may be classified into three categories, which are based respectively on phase separation, *e.g.* Baker-Williams' method,¹⁾ adsorption, *e.g.* Mark-Saito's method,²⁾ and molecular sieving, *e.g.* gel permeation chromatography (gpc).³⁾

Recently Belenkii and Gankina,⁴⁾ Otocka and Hellman,^{5,6)} and the present authors^{7,8,9)} independently found that tlc can be applied to fractionation of homopolymers according to molecular weight, as well. The explanation of its separation mechanism is, however, somewhat different for different authors. Adsorption-

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desorption process is regarded as the cause of allowing fractionation.⁵⁾ Molecular sieving effect is considered also to play an appreciable role in fractionation process.⁴⁾ On the other hand, we have pointed out that the separation by molecular weight could be attained dominantly through phase separation phenomena that take place on chromatoplate.^{8,9)}

Two specific observations made in our experiments⁸⁾ are noteworthy. One of these is that fractionation by tlc was achieved at constant temperature with solvent mixtures of fixed composition as developer. In other words, separation by molecular weight was attained without making any gradient in either temperature or composition of developer. This is in sharp contrast to the Baker-Williams method,¹⁾ in which fractionation is caused by composition and/or temperature gradients. The other observation is that on developing polystyrene with a mixture of acetone and benzene, values of R_f (rate of flow) increased with increasing the content of benzene (nonpolar solvent) in the mixture. This result is also in conflict with a general rule established in adsorption tlc¹⁰⁾ such that sample migrates lower on chromatogram when solvent polarity is reduced.

The findings mentioned above appear to imply that the fractionation mechanism in tlc cannot be interpreted uniquely in terms of any one of three known processes, namely, phase separation, adsorption, and molecular sieving. For the explanation some unknown processes other than the above need to be taken into account. The purpose of the present work is to explore what these unknown processes are, and thereby to establish a general rule for finding experimental conditions capable of fractionation by tlc.

II. PREVIOUS OBSERVATIONS CHARACTERISTIC OF FRACTIONATION BY TLC

Since we had a success in fractionating a polystyrene sample by tlc,^{7,8)} we have investigated what actions are operative to allow fractionation of polymer by difference in molecular weight. In the following we will briefly describe some results⁹⁾ that had been observed before the present work was started.

Figure 1 indicates variation of R_f -values obtained for an isotactic poly(methyl methacrylate) sample, with changing composition of a developer system consisting of chloroform (good solvent for this polymer) and methanol (nonsolvent with high polarity). On the other hand, we will show a chromatogram obtained for isotactic poly(methyl methacrylate) samples having different molecular weights with a chloroform-methanol mixture of a low methanol content ($v_2=0.05$ in volume fraction) as developer (see Fig. 2). The molecular weights of samples tested are listed in Table 1. In this chromatogram no molecular weight dependence of R_f is observed. Referring to Fig.1 we notice that the composition of developer used for obtaining Fig.2 lies just in the range between zero and 0.1 of v_2 , where a steep increase of R_f with v_2 is observed. As seen in Fig.1, values of R_f in a region of v_2 , 0.6, decrease steeply with further increase of v_2 . When the mixture with a methanol content that falls just intermediate in the aforementioned region ($v_2=0.71$) is used as developer, a chromatogram is obtained which indicates a strong molecular weight dependence of R_f as shown in Fig. 3. Separate experiments of phase equilibrium indicated that each sample polymer gave

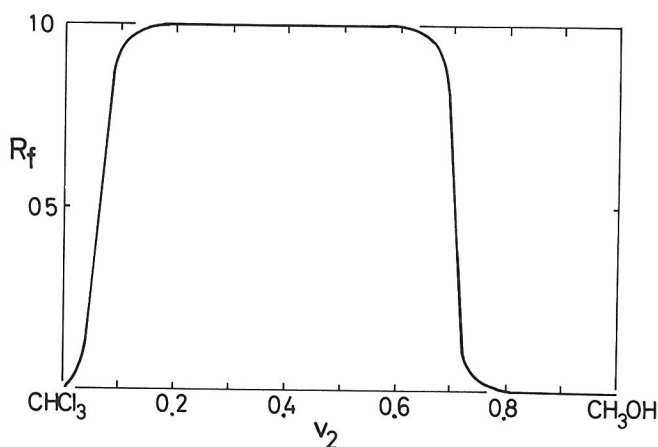


Fig. 1. Changes in R_f -value of PMMA sample, iMA-2, with composition of developer consisting of chloroform and methanol.

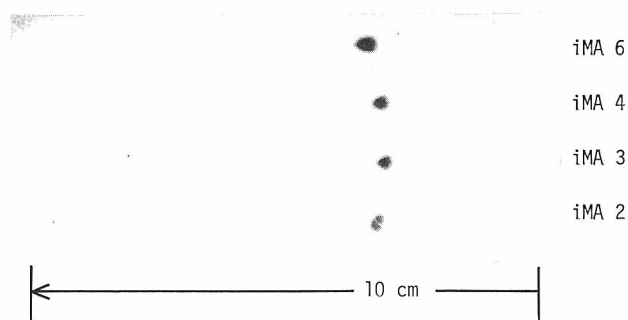


Fig. 2. Chromatogram obtained for fractions of PMMA with different molecular weight in chloroform-methanol mixture at $v_2=0.05$, volume fraction of methanol.

Table 1. Molecular Weights of Sample Polymers Applied.

Code	$M \times 10^{-4}$
Isotactic poly(methyl methacrylate) ^{a)12)}	
iMA-6	4.3
iMA-4	11.4
iMA-3	16.5
iMA-2	41.2
Polystyrene ^{b)}	
P-1	0.20
P-3	1.03
P-4	5.10

a) Molecular weights of PMMA fractions were calculated with a relation between intrinsic viscosity $[\eta]$ and $M_w^{11)}$ established in chloroform at 25°C given by

$$[\eta] = 4.8 \times 10^{-5} M_w^{0.8}$$

b) Molecular weights are weight averaged values cited from a catalog of Pressure Chemical Co., Ltd., Pittsburgh.

a clear solution with the mixture of $v_2=0.05$, whereas incipient turbidity appeared first at $v_2=0.7$, followed by precipitation of sample in the vicinity of $v_2=0.75$.

Another tlc experiment was made on six fractions of polystyrene (Pressure Chemical Co., Pittsburgh) with a mixture of cyclohexane (50 ml) and butanone

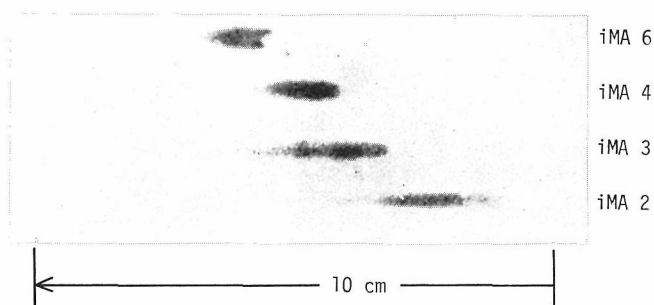


Fig. 3. Chromatogram obtained for the same fractions of PMMA as in Fig. 2 under a precipitation condition ($v_2=0.71$).

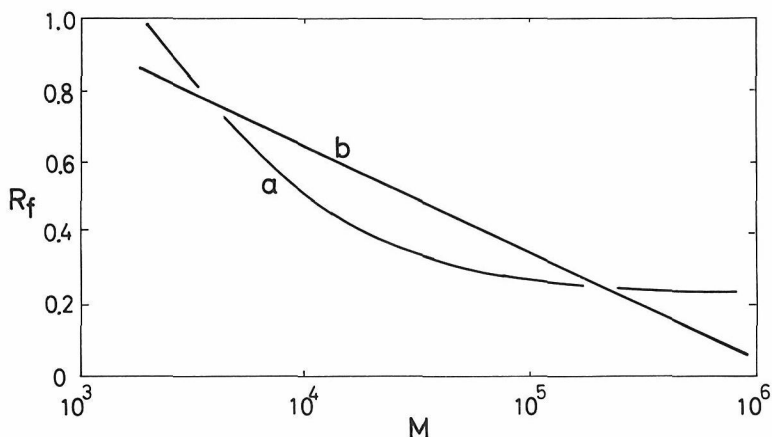


Fig. 4. Relations between R_f and logarithm of molecular weight observed for polystyrene by development with a mixture of a polar and a nonpolar solvent (line a) and with a mixture of good solvents and precipitants (line b).

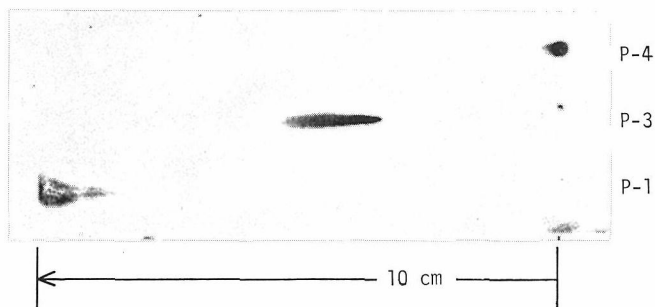


Fig. 5. Chromatogram obtained for polystyrene fractions with different molecular weight by using acetone as developer.

(2 ml) as an initial developer (see Table 1). It had been ensured that no precipitation of the samples took place in the above mixture at 25°C. A concentration gradient development was employed: 5 ml of butanone was added to the initial developer at a speed synchronized with elevation of solvent front. The result shows a molecular weight dependence that vanishes rapidly in a range of molecular weights higher than 10^6 as shown by line (a) in Fig. 4. In contrast to the above, results obtained for the same samples with a solvent mixture consisting of good and non-solvents (benzene : butanone : acetone : ethanol = 5 : 3 : 6 : 4 by volume) indicated another molecular weight dependence of R_f as such, $R_f = A + B \log M_w$, where A and B are constants (see line (b) in Fig. 4). It should be noted that the solubility of polymer in this mixture varies appreciably with molecular weight. Otocka and Hellman have also reported the similar difference between the molecular weight dependences of R_f as is seen in Fig. 4, when two different developer systems, namely carbontetrachloride + tetrahydrofuran, and acetone + toluene or chloroform, were used (see Fig. 5 in their original text⁸⁾).

The same trend as mentioned just in the above was found out again by investigating development characteristics of polystyrene samples P-1, P-3 and P-4 with acetone (see Table 1). It should be recalled that the solubility of polystyrene in acetone depends markedly on molecular weight; P-1 ($M_w = 2,000$) and P-3 ($M_w = 10,300$) are soluble at room temperature, while P-4 ($M_w = 51,000$) is no more soluble. Figure 5 shows the chromatogram obtained at 20°C. It is worth noting that sample P-3 is developed intermediate while P-1 reaches the solvent front, and R_f -value of P-3 becomes higher with elevating operation temperature, approaching 1.0 at 26°C or higher, as has been reported in our previous paper.⁸⁾ Needless to say, adsorption mechanism is never operative in this case because developers with less polarity than acetone, *e.g.* butanone, and benzene, can allow migration of the sample up to solvent front.

On the basis of experimental findings mentioned above we have concluded preliminarily that the cause of allowing fractionation by tlc is concerned mainly with phase separation process. However this conclusion does not exclude completely the possibility of polymer fractionation due to adsorption-desorption process. We want to emphasize here that tlc development due to phase separation process is much more efficient for the purpose of polymer fractionation than that by adsorption-desorption process, as clearly demonstrated in Fig. 4 as well as by the result of Otocka and Hellman cited above.⁸⁾

III. A CONSIDERATION ON PHASE SEPARATION IN TLC

First we will generally consider factors that govern phase equilibrium in polymer solution. The process of fractionation involves a phase separation followed by a partition of polymer species between two phases according to molecular weight. Thus the factors are the molecular weight M , the interaction parameter between polymer and solvent χ , and the polymer concentration v_p (expressed by volume fraction) at a constant temperature.¹³⁾ Hence there are two different ways that lead a given system to phase separation. One of them is to make χ larger than 0.5, for instance, by changing the composition of solvent mixture. The other way is to increase v_p

by some means, but this is valid for cases where χ is already a little bit higher than 0.5. When χ increases with v_p , phase separation in this way is attained more effectively.

Now turning back to our problem, we will discuss these two ways giving rise to phase separation from the viewpoint of tlc. Because there exists thermodynamically no way other than the above two, and still possible was fractionation by tlc, one should expect some specific effects caused during the preferential retarding process of sample by adsorbent. It is quite presumable that the composition of developer system is changed due to selective adsorption by adsorbent so that a composition gradient is produced along the direction of development. On the other hand, one may expect some change in a phase ratio along the direction of development. By the phase ratio r , we mean the weight of developer retained by unit weight of adsorbent. Such a change in the phase ratio will cause relatively to increase polymer concentration, possibly resulting in phase separation. In the following we will investigate whether or not these two changes really take place on chromatoplate.

IV. EXPERIMENTAL TECHNIQUES APPLIED

Developer system. For the sake of simplicity required for the present purpose of analysing experimental results, a binary mixture of benzene and acetone with a given composition (1 : 10 by volume) was applied. This developer system is hereafter designated as BA developer. The composition was chosen so that a monodisperse polystyrene P-4 with molecular weight of 5.1×10^4 migrates intermediate on chromatogram, that is to say, so as to close R_f to 1/3, an optimum value known in normal tlc.¹⁰⁾ The solvent systems with other compositions were also used corresponding to each experimental purpose.

Adsorbent. As the stationary phase we used a silica gel "Kieselgel G" (Merck AG., Darmstadt, Germany). Preparation of chromatoplates with this silica gel has been described previously.^{8,14)}

Tlc procedure. The development techniques have been mentioned elsewhere.^{8,14)} Here the sample size and the method of staining chromatograms should be noted. The sample size applied was always in the range from 15 to 20 μ g unless specially noted. For staining of chromatograms obtained for polystyrene and poly(methyl methacrylate) the method with thymol blue⁸⁾ and with iodine¹⁴⁾ were employed, respectively.

V. COMPOSITION CHANGE OF DEVELOPER DURING DEVELOPMENT

A direct simple method for detecting if any composition change of developer takes place during the development may be a "step-spot" procedure.¹⁵⁾ This procedure is to put several spots of a given substance on chromatoplate in such a way each spot lies on an oblique straight line as shown schematically by line (a) in Fig. 6. Suppose a development of a sample whose R_f -value increases uniquely with increase of the

polarity of developer, strictly obeying adsorption mechanism. Now we will consider a case that such a sample is subjected to the step-spot development with a developer composed of a polar and a nonpolar solvent. If no change in the composition of developer during the development takes place, each spot travelled should fall again on a straight oblique line as indicated by line (b) in Fig. 6. When the developer undergoes a partial demixing while penetrating the dry adsorbent, the composition at the solvent front will become richer in the nonpolar component than that at dip level so that a composition gradient may be produced along the direction of development. Under such a circumstance the value of R_f will be dependent on a distance between starting position and dip level, and become smaller with increasing the distance. As the result, the locus of spots travelled will form a downward convex curve as represented by line (c) in Fig. 6.

The step-spot procedure applied for a *suitably*^{*)} chosen solute, *p*-phenyl azophenol, demonstrated the effect of composition change in a developer consisting of benzene and acetone with a volume fraction of benzene of 0.9 (see Fig. 7). However, when the same developer system but with lower contents of benzene (0.5 or less) is used for development of another *suitably* chosen solute, bromocresol green, the chromatogram showed no indication of composition change (Fig. 8). These results may be interpreted in terms of a trend that the demixing will become more remarkable with decreasing the ratio of the polar solvent component.¹⁵⁾

On the other hand, Fig. 9 shows a chromatogram obtained for sample P-4 with

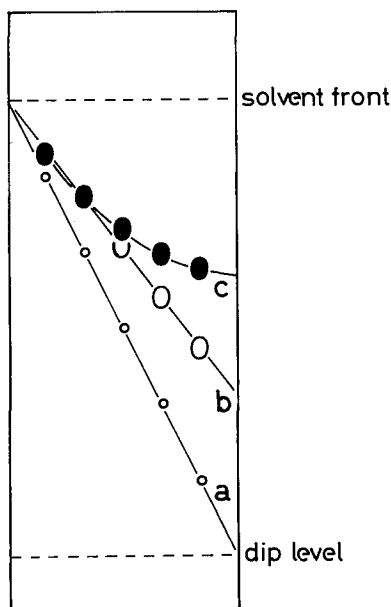


Fig. 6. Schematic demonstration of "step-spot" procedure for detecting solvent demixing on chromatoplate. For the details, see text.

^{*)} By this word it is meant that the solute is developed strictly in accordance with adsorption mechanism.

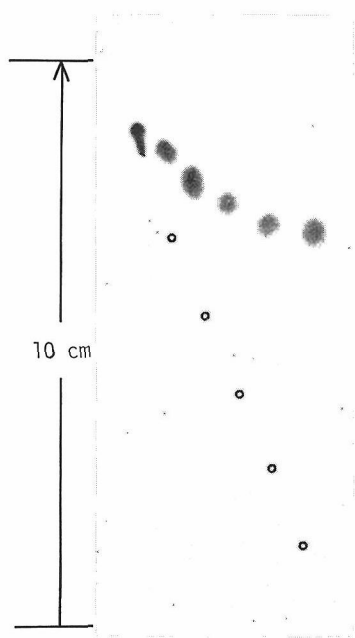


Fig. 7. Chromatogram obtained for *p*-phenyl azophenol by step-spot procedure with a benzene-acetone mixture (9 : 1 by volume) as developer. The effect of definite composition change of developer is seen.

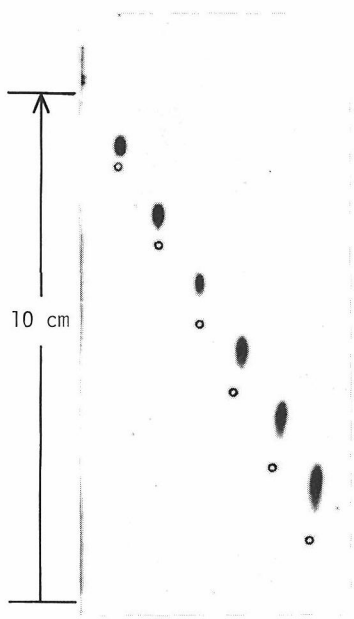


Fig. 8. Chromatogram obtained for bromocresol green by step-spot procedure with a benzene-acetone mixture (1 : 9 by volume)—BA developer. No effect of composition change of developer is seen.

the same developer system as used for bromocresol green, which indicated no composition change. As is seen in the figure, the spots travelled are falling not on an oblique line as if some composition change of developer were produced, and this is in sharp contrast to the result obtained with bromocresol green. In addition it is also noted in Fig. 9 that three spots migrate up to the same level no matter where each spot was located at the start. In this connection the result obtained with a single solvent (acetone), which was given already in Fig.5 and had no concern with the composition change of developer, should be referred to again. This feature confirmed by the different developments suggests the importance of another cause that may dominate polymer fractionation by tlc, namely the phase ratio.

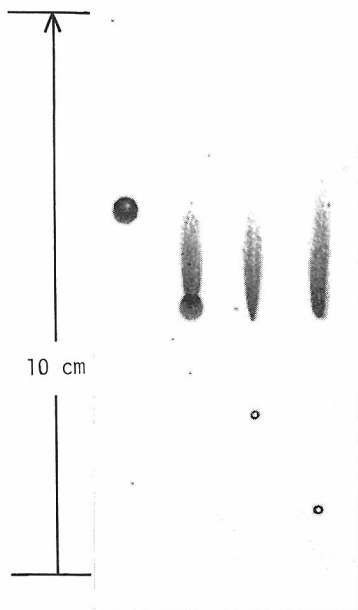


Fig. 9. Chromatogram obtained for polystyrene sample, P-4, by step-spot procedure with BA developer.

VI. INFLUENCE OF CHANGE IN PHASE RATIO UPON PHASE SEPARATION

The phase ratio r has been determined as a function of the position of solvent front z by using plastic sheets, on which silica gel is coated (Tokyo Kasei Co., Ltd., Tokyo). This sheet can readily be cut to any size. The determination was carried out for two developing techniques, *i. e.* the ascending and descending technique. Dimethylformamide (DMF) was employed as developer to minimize errors due to evaporation. The procedure taken for the determination was the same as is usually made in paper chromatography.¹⁶⁾ Changes in the phase ratio with varying the position of solvent front (hereafter referred to as the solvent concentration profile) are illustrated in

Fig. 10. From the figure one sees that the solvent concentration profile is different for different development technique, and the phase ratio observed at an intermediate value of z in the ascending development is much higher than that in the descending development. Thus we may expect that chromatograms obtained for polystyrene with different development techniques should be different from one other if the change in the phase ratio be a dominating factor for fractionation.

In this view, a descending development of sample P-4 has been performed with BA developer, which had been known to give intermediate R_f -values for the above sample when the ascending development is applied. No migration of the sample was observed. The reason may be attributed to too low phase ratios comparing to those

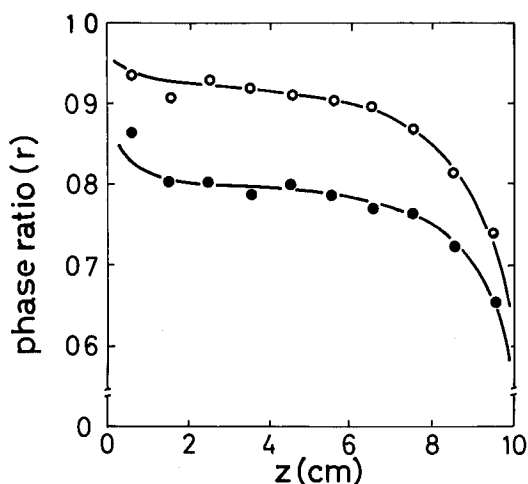


Fig. 10. Solvent concentration profiles observed with different development techniques: Open and filled circles are referred to the ascending and descending technique, respectively. For the detail, see text.

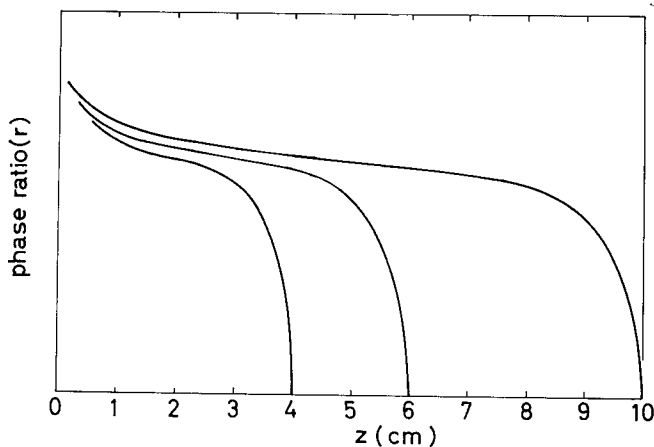


Fig. 11. Schematic description of solvent concentration profiles expected for different levels of solvent front (z_t).

in the ascending development as clearly indicated in Fig. 10. This finding allows us to assume that the phase ratio is related closely to the migration of sample polymer. In other words, a sufficient amount of developer should be necessary to dissolve the sample for permitting it migrate. The same reason as the above may hold for explaining the chromatogram shown in Fig. 9.

The above observation allows us to remember a rule prevailing in paper chromatography. To explain the rule, Fig. 11 is given, in which changes in the solvent concentration profile with different levels of solvent front are drawn schematically. Now we may describe the rule in such a way that three profile curves shown in the figure can be superposed to a master curve when a ratio of z/z_f is taken as the scale of ordinate instead of z , where z_f is the level of solvent front observed at a given moment. Taking this rule into consideration we further investigated the development characteristics of sample P-4 by applying the step-spot procedure to cases where the value of z_f is different. Two runs of the development with BA developer were carried out for different values of z_f , *i.e.* 4 and 6 cm. The chromatograms thus obtained are shown in Fig. 12 and 13, respectively. By analysing these chromatograms, and that given already in Fig. 9 ($z_f=10$ cm), one can deduce that the spots travelled afford approximately the same R^*_f -value of 0.49, where R^*_f is defined tentatively as:

$$R^*_f = (\text{distance between level of spot travelled and dip level})/z_f$$

It should be emphasized here that the R^*_f -value is independent of z_f . In other words, values of R^*_f are the same for the same phase ratios. This implies, in turn, that there will be a threshold in the magnitude of phase ratio for initiating migration of sample polymer.

At this stage it is worthwhile to estimate the concentration of polymer sample when migrating on chromatoplate. For this purpose knowledges of the spot area, the sample size, and the phase ratio of developer are necessary. The solvent con-

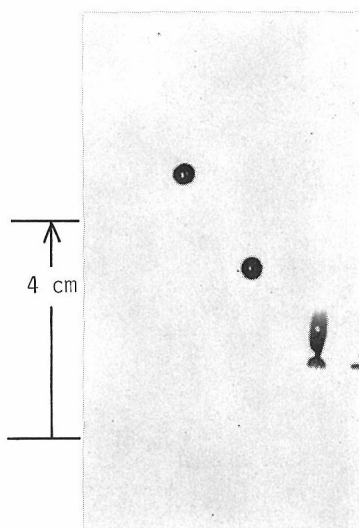


Fig. 12. Influence of z_f on migration of sample observed for P-4 by step-spot procedure with BA developer ($z_f=4$ cm).

centration profile of DMF shown in Fig. 10 was used, though the situation for BA developer might not be entirely the same. The calculation was made by ignoring the concentration distribution within the periphery of spot travelled. The polymer concentration in spots shown in Fig. 9 was reckoned to be about 0.01 in volume fraction, which is approximately of the same order as $v_p=0.03$ observed by a separate phase equilibrium experiment made with the same solvent system at 20°C (see Fig. 14).

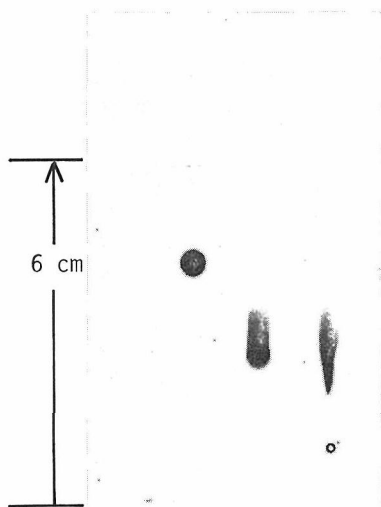


Fig. 13. Chromatogram obtained for the same purpose as Fig. 12 with the same sample and developer ($z_f=6$ cm).

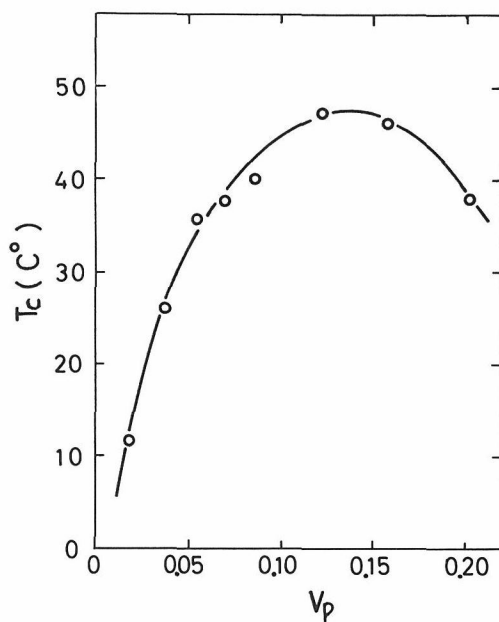


Fig. 14. Phase diagram for a polystyrene fraction, P-4, in BA developer.

On the basis of observations made for the phase ratio and the R_f^* -value we may draw a conclusion that in fractionation by tlc, phase separation phenomena due to the increase in polymer concentration play the dominant role. Understanding tlc as the process that sample components are preferentially retarded by the adsorbent as carried past by the mobile phase, we may elucidate the fractionation mechanism such that phase separation *retards* migration of each component of sample polymer at different rate which will increase with its molecular weight, thus giving different R_f -value for each component.

VII. SHAPES OF SPOTS TRAVELLED AND PHASE SEPARATION

During the course of this study we have observed some distinct characteristics of the shape of spots travelled. Polymer samples with sufficiently narrow molecular weight distributions were found to exhibit always a peculiar spot shape that may be expressed by phrases, a "diffusing-up" at the head of spot, and a "clearly cut off form" at the tail of spot, when development proceeds involving phase separation process. This shape of spots is seen in Fig. 3, 5, and 9, and can be distinguished obviously from that observed in developments in which adsorption alone is operative (see Fig. 2 and 15). Such a spot shape appears regardless of the difference in the sort of developer used, the molecular weight, and probably, the chemical constitution of sample polymer.

As to the influence of polymer amount spotted on chromatoplate (sample load) a more detailed description is necessary. A chromatogram obtained for sample P-4 with a benzene-acetone mixture (3 : 40 by volume) under variation of sample load is shown in Fig. 16. Inspection of the chromatogram reveals two features: [1] the level of spot tails is located lower with increase in sample load; [2] the head of spots appears to reach a common level independent of sample load.

In advance of discussing the above findings, we will consider briefly the situation of phase separation that would take place within the periphery of a spot after macromolecules involved in the spot were faced to penetration of the mobile phase at a constant temperature. For the sake of simplicity it is assumed that a single solvent whose interaction parameter χ exceeds 0.5 and a sample polymer with no polymolecularity are applied. First a phase equilibrium is attained by which the macromolecules are partitioned between two phases, a dilute (sol) and a more concentrated (gel) phase. It follows that macromolecules in the sol phase will be carried past by

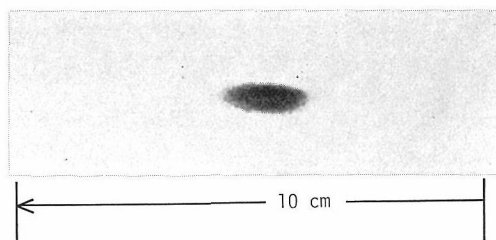


Fig. 15. Typical shape of spot travelled on chromatogram obtained in adsorption-desorption process; sample P-4 and a mixture of 2-butanone and cyclohexane (1 : 6 by volume) are applied.

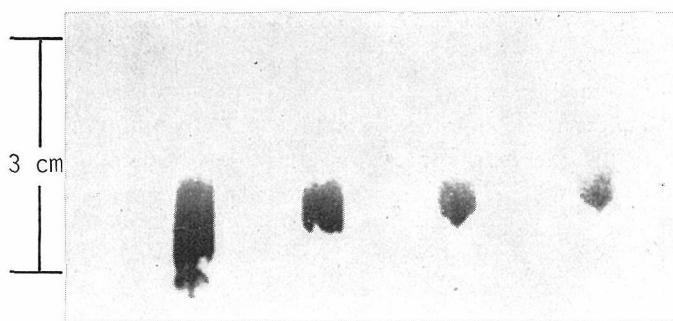


Fig. 16. Effect of sample load upon shape of spot travelled observed for sample P-4 with a benzene-acetone mixture (3 : 40 by volume). Sample loads are, in order from left to right, 40, 30, 20, and 10 μ g, respectively.

the mobile phase while those in the gel phase will be strongly retarded to migrate. This situation will, at least, account for the reason why the diffusing-up effect is generally observed, differing from developments in which adsorption alone is operative.

When the sample load is very much high, the retardation action mentioned above would become pronouncing so that a certain fraction of macromolecules in the gel phase remains practically without migration. Such a speculation might explain the feature [1] observed when the influence of sample load was investigated (see Fig. 16). The extent to which the diffusing-up effect is detected visually may be dictated by the spontaneous decrease in phase ratio in the development direction, and by a decrease in the polymer amount carried past by the mobile phase, which may be caused by polymer adsorption onto adsorbent. This might give an elucidation for the other feature [2].

The above interpretation given for the spot shape is still qualitative but may be plausible, at least, from the standpoint of phenomenological situations that really hold during the development on chromatoplate. On the other hand, the spot shapes often seen in adsorption chromatography (see, *e.g.* Fig. 15) have been argued on some theoretical bases such as the delay in establishment of adsorption equilibrium, the nonlinearity of adsorption isotherm, and the diffusion effect.¹⁷⁾ Thus for a complete understanding of the spot shapes in chromatography due to phase separation it is obvious that one should take also the effects just aforementioned into consideration.

VIII. OTHER FACTORS AFFECTING R_f -VALUES

Molecular sieving effect. Tlc development is concerned generally with a variety of factors and their combinations.¹⁸⁾ Among these, the effect of molecular sieving upon tlc has been investigated by Halpaap and Klatyk.¹⁹⁾ They found some peculiar phenomena concerning the relation between the pore size of silica gel and the development characteristics. Belenkii and Gankina have studied also the effect from the standpoint of polymer fractionation by tlc.⁴⁾ When the report of the latter authors⁴⁾ is reread, one finds, despite their emphasis, that silica gel (designated MSA-1) with large pores which will be accessible to macromolecules exhibits no indication of the

molecular sieving effect. In this connection we have also experienced that tlc developments effected by both adsorption and phase separation gave completely different chromatograms due to a slight change in temperature. Thus to prove whether or not the molecular sieving effect really acts in tlc, one should collect much more observations made with various adsorbents of different pore nature, and under a specified condition such that a certain developer system with constant composition is commonly employed at constant temperature.

Effect of polymer concentration. It has already been well known that when sample load is inappropriately high, R_f -value often deviates from that found with a suitable load.¹⁸⁾ Therefore a need for adjusting the amount of sample to avoid higher loads is apparent. An effect relating to this problem has been discussed in a previous section of this paper (see Fig. 16). During the course of our works on tlc, we have observed frequently upward or downward tailing phenomena on chromatogram especially when a developer used was not well matched in the whole chromatographic system and/or the sample load was too much high. On the other hand, Belenkii and Gankina implied that tailing phenomena could be attributed partly to the molecular sieving effect.⁴⁾ However we believe, at present, that the interpretation given by the above authors finds little favor. In this connection it is noted that we are working usually with sample loads of around 15 μ g so that no serious error due to the sample load effect will be coherent in final results.

IX. CONCLUDING REMARKS

In summary we will briefly note some main features in fractionation by tlc found by the present study. The most essential feature is demonstrated by two development experiments in contrast, which are made for polystyrene (nonpolar polymer) having a molecular weight higher than 5×10^4 , respectively with benzene (nonpolar, good solvent) and with acetone (polar nonsolvent) as developer. The development with benzene allowed migration of polymer up to the solvent front; whereas that with acetone no migration. This simple and distinct difference in the development characteristics can never be interpreted in terms of a separation mechanism in which solely adsorption-desorption process is operative. One has, therefore, to attribute the cause of fractionation by tlc to phase separation phenomena that will retard migration of polymer at different rate which increases with the molecular weight—precipitation chromatography.

When mixtures of benzene and acetone are used as developer, a composition change takes place along the direction of development due to selective adsorption of acetone by adsorbent. The composition gradient thus produced is much pronounced if the acetone content in mixture is lower than ca. 0.2 in volume fraction. Under use of the mixtures with such compositions fractionation can be achieved but ceases to be efficient in the range of molecular weights higher than 10^5 . This is the second feature.

The third feature is that the amount of developer retained in adsorbent (phase ratio) is lowered appreciably with increase in the distance from dip level. As the result, the polymer concentration on chromatoplate reaches its critically miscible limit at a certain distance from dip level so that polymer ceases to migrate at this

level. This situation may be considered as the cause by which fractionation most effectively occurs.

The last to be mentioned concerns the properties of solvents used as developer, which may, for the present purpose, be classified into two categories: The one is the thermodynamic nature relative to sample polymer and the other is the solvent strength¹⁰⁾ in a sense of the eluotropic series. Solvent systems appropriate for fractionation must dissolve sample polymer, at least, but be so poor that phase separation takes place caused by a slight change in polymer concentration. However, the dissolving power is only a necessary condition but not a sufficient condition. The solvent strength required as developer must be high enough to overcome interactions between polymer and adsorbent¹⁴⁾. In this connection the observation of Otocka and Hellman that carbontetrachloride could not allow migration of polystyrene should be mentioned.⁵⁾ This nonpolar solvent is good solvent for the polymer but has a too low solvent strength as the developer.

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